

10/511818

P1 1019253

REPTO 1 OCT 2004

REC'D 16 JUN 2003

WIPES DCI

# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE  
United States Patent and Trademark Office

June 09, 2003

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 60/374,715

FILING DATE: April 23, 2002

RELATED PCT APPLICATION NUMBER: PCT/US03/12602

By Authority of the  
COMMISSIONER OF PATENTS AND TRADEMARKS



*M. Sias*  
M. SIAS  
Certifying Officer

PRIORITY DOCUMENT  
SUBMITTED OR TRANSMITTED IN  
COMPLIANCE WITH  
RULE 17.1(a) OR (b)

CERTIFIED COPY OF  
PRIORITY DOCUMENT

## PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(b)(2).

Docket Number: STRM 8363US

## INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY
Strahilevitz	Meir		Seattle, Washington

## TITLE OF THE INVENTION (280 characters max)

METHOD FOR IMPROVED TARGETING OF LIGANDS

## CORRESPONDENCE ADDRESS

CUSTOMER NUMBER 001688

POLSTER, LIEDER, WOODRUFF & LUCCHESI, L.C.  
763 South New Ballas Road  
St. Louis, Missouri 63141

STATE	Missouri	ZIP CODE	63141	COUNTRY	U.S.A.
-------	----------	----------	-------	---------	--------

04/23/02

JC996 U.S. PRO

JC996 U.S. PRO  
60/374715

## ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification  
☐ Drawings

Number of pages [17]  
Number of sheets [ ]

☒ Claiming Small Entity Status  
☐ Other (specify)

## METHOD OF PAYMENT

A check or money order in the amount of \$80.00

☐ \$160.00 for large entity  
☒ \$80.00 for small entity

is enclosed to cover the Provisional filing fees.

The Commissioner is hereby authorized to charge any additional fees or credit overpayment under 37 CFR 1.16 and 1.17 which may be required by this paper to Deposit Account 162201. *Duplicate copies of this sheet are enclosed.*

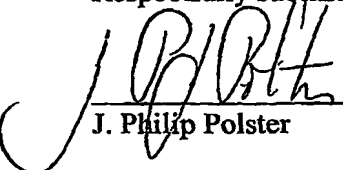
---

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No  
☐ Yes, the name of the Government Agency and the Government Contract Number are:

---

Respectfully submitted,

  
J. Philip Polster

Date: 4/23/02


Reg. No. 24,739

Express Mail No.: EL 820488385 US

**EXPRESS MAIL FILING CERTIFICATE**

RE: U.S. Patent Application  
TITLE: METHOD FOR IMPROVED TARGETING OF LIGANDS  
INVENTOR: Meir Strahilevitz

I hereby certify that this U.S. Patent Application is being deposited with the United States Postal Service utilizing the "Express Mail Post Office to Addressee" service addressed to Commissioner for Patents, Washington, D.C. 20231 on April 23, 2002.

  
\_\_\_\_\_  
J. Philip Polster

4/23/02  
\_\_\_\_\_  
Date of Signature

Express Mail No: EL 820488385 US

**METHOD FOR IMPROVED TARGETING OF LIGANDS WHEREIN THE LIGAND COMPRISES A TARGETING MOLECULE, TO A SITE IN AN ORGANISM, PARTICULARLY A CANCER SITE, COMPRISING AFFINITY BINDING OF AT LEAST ONE OF THE GROUP COMPRISING OF TUMOR CIRCULATING ANTIGEN, ANTIBODY SPECIFIC TO TUMOR ANTIGEN, COMPLEX OF TUMOR CIRCULATING ANTIGEN AND ANTIBODY TO TUMOR ANTIGEN AND ANTIBODIES SPECIFIC TO AT LEAST ONE CONSTITUENT OF THE LIGAND**

The field of the invention is targeting of ligands to a site in an organism, particularly a cancer site, by utilizing adsorbents with selective or specific affinity to chemical species, wherein the adsorption of at least one molecule is associated with improved targeting to the site in the body, such as a tumor site and increased concentration of the ligand in the targeted site, relative to its concentration in the rest of the body.

The adsorbents are primarily incorporated in an extracorporeal device, but the field of the invention is not limited to Extra Corporeal Affinity Adsorption or Extracorporeal Affinity Dialysis (Extracorporeal Affinity Dialysis is a method involving both dialysis (and/ or filtration) and adsorption as detailed in US patent 5,753,227, see in particular; column 5 line 40 to column 7, line 44), in that at least one of the adsorbents may be administered to the organism, such as by intravenous intraperitoneal, or other route and by binding of the "adsorbent" to the species, the species is cleared faster than it would otherwise clear from the body of the organism by increased elimination from the body, such as elimination through the kidney or liver or Reticulo Endothelial System (RES), or by increased metabolism and breakdown or neutralization of the chemical species.

The methods and devices that are describing the field of the invention and are relevant to elements of the invention that is the subject matter of the current application, include the following patents, patent applications and publications, all of which, including the references cited in said documents are incorporated herein by reference: US patents 4,375,414 and US 4,813,924 and any and all divisional applications or patents of said patents; US 6,039,946 and US 5,753,227 US 6,264,623 and published US patent application US 2001/0039392A1, US 2002/0019603 A1 and all their US divisional applications and issued patents and all equivalents and foreign counterparts of said patents and the divisional patent applications and divisional patents of said patents. PCT WO96/37516 and its foreign and US counterparts and all divisional applications thereof. For abbreviation all these patents and patent applications will be referred to at times as "my patents". Also incorporated herein by reference are the following patents and publications, including the references cited in them: Nillson et al: US patent US 6251,394 B1, V Pimm: Nucl. Med. Biol. Vol. 22, No 2, pp. 1020-1027, 1993, D. A. Goodwin et al: Antibody Immunoconjugates and Radiopharmaceuticals, Vol. 4, number 4, pp. 427-434, 1991, Van Kroonenburgh et al., Nucl. Med. Commun., Vol. 9, pp. 919-930, 1988, M Gurkavij et al., Cancer Research (Suppl.) Vol. 55, pp. 5874s - 5880s, December 1, 1995. The limitations inherent in methods known to date, for the targeting of ligands, such as Treatment Ligands (TL) and Visualization Ligands (VL) [The definition of these terms is in accordance with PCT application WO 96/37516 its US and foreign counterpart and their divisional applications.], to a site in an organism, particularly, but not exclusively tumor site. As known in the art of targeting ligands to sites in the organism, such sites may be for example, an area of infarct in the heart, or a

ligands to sites in the organism, such sites may be for example, an area of infarct in the heart, or a specific organ such as the Thyroid gland, as examples. The tumor sites, in accordance with the current invention are both solid tumors that are tumors originating from cells outside the blood and the bone marrow (e.g., Leukemias and Lymphomas) as well as "soft tissue tumors" such as Leukemias and Lymphomas. Examples of solid tumors are well known and include, for example, colon carcinoma ovarian carcinoma pancreatic carcinoma as well as sarcomas, as well as brain tumors, such as Astrocytoma and Glioblastoma. The treated organism which is a mammal, including, but not limited to a human, is conceptualized in accordance with the present invention to contain three compartments: The Tumor Compartment (TC), The Blood Compartment (BC) and the rest of the organism: The Healthy Tissue and Healthy Organs Compartment (HC), including for example, the liver, kidney, spleen and lymph nodes. It should be realized that some or all of the organs may include tumor cell masses (such as metastases of tumor). As used in the current invention the non tumor tissue and cells of the organ (kidney and liver for example) will be referred to as HC and the metastatic cells (typically in the form of multiple cells): are referred to as TC, even though they reside in the kidney or the liver, for example. It is clearly appreciated that the three compartments are in fluid communications, through, but not limited to the interstitial fluid (IF), the capillaries of the blood circulatory system and the fluids of the Nervous System, Cerebro Spinal Fluid (CSF) and peritoneal fluid (PF), for example. Further more, there is also fluid communications between the intracellular fluid (ICF) and the other compartments, including the above fluid compartments, for example the IF. It is also realized, that various chemical and cellular species, such as proteins, peptides, various antigenic and haptenic molecules, whether endogenous to the organism, or administered to the organism (such as TL and VL) various sub populations of lymphoid cells and macrophages, invading microorganisms such as viruses, bacteria and protozoa, are able to move from one compartment to the other, and may be at a steady state balance between the various fluid compartments and TC, BC and HC, meaning that removal of a molecular or cellular species from the BC, may for example change the rate of movement of the removed species from the other compartments to the BC.

In accordance with the present invention the tumor cells of the present invention contain Tumor Antigens (TA) and the Blood Compartment (BC) may contain Circulatory Tumor Antigens (CA). It will be realized in accordance with the discussion above, that the CA, being a chemical species, usually will be able to migrate between the various compartments. A CA may be identical in chemical and antigenic structure to TA or it may have chemical and antigenic structure that is similar to but not identical to TA (for example the CA may have different affinity to specific monoclonal antibodies specific to TA then the affinity of same antibodies to TA). The body of the organism may produce antibodies to the TA and/or the CA. These antibodies are produced by the organism, as the result of the immune system in the organism mounting a humoral immune response directed at the TA and/or the CA. These antibodies are referred to as Native Antibodies (NAB). NAB may act as "Enhancing Antibodies" (K. A. Hellstrom and I. Hellstrom: In: Encyclopedia of Immunology, I. M. Roitt and P. J. Delves Eds. Academic Press, 1998 p. 2440-2445), such antibodies may bind to TA and mask the TA on the tumor cells, they may bind to lymphoid cell receptors and inhibit their ability to kill tumor cells, or they may participate in inducing suppressor lymphoid cells that inhibit tumor killing by Cytotoxic T cells (CTL). Examples of TA are given in WO 96/37516 and include Carcino Embryonic Antigen (CEA), Le(y), Alpha-Fetoprotein (AFT). Many other tumor antigens which include tumor specific antigens and developmental antigens (for example ovarian carcinoma CA-125 antigen) as well as monoclonal antibodies specific to these antigens are known in the art. (B. J Van

den Eynde and A. M. Scott: in Encyclopedia of Immunology , Supra pp. 2424-2430 .) In the presence of both CA and NAB , at least some of the CA and NAB bind to each other and produced CA-NAB complex . Such complexes may be in "antigen excess" (CA2NAB) , wherein the complex will generally have free antigenic sites available to bind additional antibody molecules, or it may be in "antibody excess" (CANAB2) , wherein the complex will generally have free antibody binding sites available to bind additional antigen molecules. CA-NAB , was found to play a role in the etiology and pathogenesis of cancer (F. A. Salinas and M. G Hanna , Jr. Eds. Contemporary Topics in Immunobiology, Vol. 15 :Immune complexes and Human Cancer, Plenum Press , 1985) and removal of CA-NAB by plasma exchange can be therapeutic (Immune complexes and Plasma Exchange in Cancer Patients, B. Serrou and C. Rosenfeld , Elsevier/ North-Holland Biomedical Press , 1981)

Factors that interfere with targeting TAB-bound VL or TL to a tumor site in an organism include:

- (1) Presence of CA or CA2-NAB, that competes with the TA for binding of the TAB.
- (2) TAB( bound to VL/TL) or CA-TAB ( bound to VL/TL) complex binds to receptors such as Fc Receptors. If TAB is toxic, for example , is radioactive or is a toxin or toxic drug, this will lead to increased concentration of toxic VL/TL in healthy organs such as the liver , by binding of the TAB's Fc to Fc receptors on liver cells, when TAB contains Fc , such as when TAB is an intact antibody ( it should be realized as mentioned earlier that TAB as defined in the current application may also be an antibody fragment , including synthetic fragment and fragment produced by genetic engineering. Complexes of such fragments with CA , even though they do not have Fc fragment, and do not bind to Fc receptors in the liver, they can still be cleared in the RES in the liver and elsewhere in Reticulo Endothelial System (RES) , by RES cells, such as macrophages.
- (3) NAB compete with TAB for binding to TA and may inhibit TH1 Helper Cells' immune response to the cancer.
- (4) CA-NAB complex concentrates in the RES, liver and kidney , for example , within the HC and toxic effects on normal organs is induced by this concentration.
- (5) Particularly , following repeated administration of heterologus targeting antibody (eg: mouse monoclonal targeting antibody to human recipient) , the recipient may produce antibodies specific to the targeting antibodies . Such antibodies may be specific to the Fc part of the antibody (antiisotypic antibodies) or they may be directed to the Fv fragment (antiidiotypic antibodies) Such anti-targeting antibody antibodies (ATAA) can be produced by the organism, also when the TAB is a chimeric or humanized monoclonal antibody (M. V. Pimm Supra , Van Kroonenburgh et al., Supra.) ATAA may compete with TA for the binding of TAB.

It should be realized that depending on the particular cancer and the individual case, CA, CA-NAB complex , NAB, free ATAA TAB-ATAA complex (after the administration of TAB) may be present in various concentrations in the BC HC and TC. Optimally, particularly when these various concentrations are not known , it may be advantageous to remove or otherwise reduce more then one of the above species, and at least in some situations it may be desirable to remove as many of these species , as possible from the BC and/or HC and/or TC, prior to the administration of the TAB-VL or TAB-TL. Reduction of the amount of one or more of the above species in the BC and/or HC and/or TC, prior to the administration of the TAB-VL or TAB-TL , can preferably be achieved , in accordance with the present invention by Extracorporeal Adsorption , but may include , in some situations , reduction of one or more of the above species, by the administration to the subject of agents that would increase the breakdown and /or clearance from the body compartments of one or

more of the above species, that are known to increase such clearance from the organism (see , for example, D. A. Goodwin, Supra).

One approach that was used to reduce this interference is the administration of unlabeled targeting antibody prior to the administration of the labeled targeting antibody. This approach was associated with limited and various success (see for examples: M Helma et al. Cancer Immunol. Immunother. (1998) 47: 39-46, Beatty, B. G. et al. Cancer Res. 49, 1587-1594, 1989., Ahonen et al.: Acta Oncologica (1993), 32, 7/8 , pp. 723-7., Schrijvers A. G. H. et al.: J Cancer Research, (1993) 53, 4383-O4390, September 15, 1993. Clearly significant improvement in specificity (e.g. : to target cell , target tissue or organ, relative to the rest of the body) of targeting is needed. Pre administration of unlabeled targeting antibody ,while binding to circulating antigen or to CA2NAB in the blood circulatory system ( or in other biological fluid such as for example, cerebrospinal fluid (CSF) and peritoneal fluid) and thus reducing the amount of subsequently administered labeled antibody in the blood and /or in the liver , by reducing the amount of circulating antigen that can bind the labeled antibody, the pre administration of unlabeled antibody will also lead to the unlabeled antibody binding to the TUMOR ANTIGEN ON TUMOR CELLS and by this mechanism will REDUCE targeting to later administered labeled antibody to the tumor target .

Thus, generally Extracorporeal Adsorption (ECA) is preferred , in view of the fact that such a method is not associated with administration of additional species to the subject (that may have their own toxicity) and is not associated with increased concentration of TAB, TL or VL in healthy organs of the subject, associated with clearance, such as the liver and kidney. Generally the removal of one or more of the above species is completed 15 minutes to 48 hours prior to the administration of the TAB-VL or TAB-TL. It should be realized that the Targeting Ligand may be a species that is not an antibody species and its binding to the targeted site in the organism , may be based on none immunologic binding, as detailed and described in the incorporated references, in particular patents 6,039,946 and 5,753,227 and their counterparts and in Pimm , Supra, Gurkavich, Supra and Goodwin, Supra. Thus the targeting ligand may be a ligand that binds none immunologically to a receptor such as Epidermal Growth Factor Receptor, or it may be the peptide hormone Somatostatin , that binds to the Somatostatin Receptor , which is present in high concentration in many tumors. The targeting ligand can also be other targeting proteins or peptides (OTP) , or they can be none peptide targeting molecules such as a drug, for example Atenolol that binds to Beta-Adrenergic Receptor and Haloperidol, that binds the Dopamine 2 receptor

My US patents and patent applications Supra and in particular PCT applications WO 96/37516 provide for improved targeting over the previous art, by including a step of ECA of a species comprising circulating tumor antigen circulating tumor antigen-antibody complex and circulating (such as enhancing) antibodies specific to tumor antigen.

These methods will not be associated with competition of the administered unlabeled antibodies with binding of the later labeled antibodies to the tumor antigen at tumor sites . This methods will also not include the risks and undesirable effects of administering of unlabeled antibody to the organism, (infection , reaction to foreign protein Immune complex Disease). Optionally the methods of my patents Supra can also add a step of removing of the targeted treatment or diagnostic ligand by



extracorporeal affinity adsorption at a predetermined time following the administration of the treatment ligand or visualization ligand to further improve targeting.

ECA removal of targeted ligands is disclosed in US patents 6,046,225 and 6,251,394 and PCT application WO 96/37516.

The removal of circulating immune complexes can be achieved in accordance with my patents supra by specific extracorporeal immunoabsorbents such as anticomplex antibodies, or by Protein A affinity adsorbents (Fresenius Immunosorba®, Fresenius Prosorba®, Fresenius C1q extracorporeal adsorbent Miro®, Kaneka Selesorb® as well as Asahi, as disclosed in US patent 4,627,915 as examples. Enhancing tumor antibodies can be removed for example, using affinity adsorbents such as tumor antigen, Protein A Immunosorba®, Prosorba®, for example, C1q, (Miro®, for example) as well as use of the adsorbents as used in Selesorb® and the above Asahi patent. Removal of circulating free tumor antigen (not complexed with antibody) can be achieved by the use of adsorbents such as specific antibody to tumor antigen.

It will be realized that tumor targeting inhibitor factors (TIF) may exist in complexes containing more than two molecular species. Such complexes may be affinity labeled for adsorption or adsorbed in the ECA column, by an affinity label or affinity adsorbent specific to any of the components of the complex, or specific to epitopes that are specific to the complex. Examples of such complexes are: CA-TAB-ATAA and NAB-CA-TAB. For example, the complex CA-TAB-ATAA may be adsorbed by antibody to any of its three components, bound non-covalently (e.g., by ligand) to Protein A that is bound covalently to the matrix in the ECA column, or it may be adsorbed by Biotinylated antibody that is bound to Avidin in the Avidin ECA column. Similarly, the CA-TAB-ATAA can be affinity labeled for adsorption, by administering to the subject a Biotinylated antibody, specific to any of its three components, to enable its specific adsorption in the ECA Avidin column or affinity labeled by antibodies to any of the components of the complex (or antibodies to epitopes specific to the complex) and adsorbed by ECA on a Protein A column.

#### EXAMPLE 1

##### STEP ONE OF EXTRACORPOREAL REMOVAL OF CA-NAB, AND/OR NAB AND/OR ATAA PRIOR TO ADMINISTRATION OF TAB-VL OR TAB-TL

Protein A-Sepharose CL-4B obtained from Pharmacia LKB Biotechnology and swollen and washed in accordance with the instruction (Affinity Chromatography . Principles and Methods, Pharmacia Biotechnology Pub., 1991), The Protein A-Sepharose is packed in a column . Preferably the column used is the commercially available Immunosorba® sold by Fresenius HemoCare Inc. Redmond, WA. The column requires the use of a plasma separator, as is well known and as recommended by the manufacturer: either a "centrifuge" type, such as Fresenius AS 104 cell separator, Cobe IBM 2997 or membrane type plasma separator, such as Kaneka Sulfox® or Cobe TPE® can be used to separate, on line, the patient's plasma from the cellular elements of blood. While the Immunosorba® column is preferred in some applications, other Protein A Adsorption columns can be used instead. When regeneration is preferred a system including two Immunosorba® columns with a Fresenius Automatic Regeneration unit, Citem 10® are used. When no regeneration of the adsorbent, column

is needed only a single Immunosorba® column can be used. When it is preferable to use a disposable column, the column utilized for adsorption can be the Fresenius HemoCare Prosorba® column in which the Protein A is covalently bound to a silica matrix. It should be realized that other Protein A columns can be used instead, using various biocompatible matrixes to which the Protein A is covalently bound. (Whenever Protein A is mentioned in this application the term includes, Peptide fragments of protein A, including synthetic peptides, that are all known in the art to be used as equivalents of Protein A). The matrixes used include natural polymers, such as cellulose and dextran, various synthetic polymers and copolymers, such as polyacrylamide, polystyrene and polyvinyl polystyrene copolymer, and silica and silicones. One suitable matrix is heparinized silicone described in D. R. Bennett et al. US patent 3,453,194.

The configuration of the matrix is also not limited to a particular form, examples of suitable forms are beads (in particular, spherical in shape), fibrous matrixes, macroporous matrixes and membranes, including hollow fibers. One possible device is that of a typical multi-hollow fiber filtration, dialysis or diafiltration design. Such a configuration consists of a bundle of hollow fibers encased in a tubular housing. The adsorbent is included in the outer space of the hollow fiber, as disclosed in US patent 5,753,194, see in particular column 8, line 28 to line 68 and figs 2 and 3, the patent uses in the example a chelator as the adsorbent, but in the current invention the adsorbent may be Protein A, either in free form, bound to a matrix, particularly by covalent chemical binding or alternatively the Protein A can be bound to the membrane, to its blood flow side to the side opposite to the blood flow or to both sides, rather than being incorporated in the outer space of the hollow fiber. The Protein A, in either free form, or matrix bound, can also be physically trapped in the membrane, (such as when the membrane is an unisotropic polysulfone membrane, for example the one produced by Amicon). When the Protein A is encapsulated it can be encapsulated in one of the microcapsules or macrocapsules as described in US patent 5,753,227, in particular column 7, line 53-65, or the double encapsulation described by Markus et al. American Heart Journal, Vol. 110 No 1, part 1, July 1985, pp. 30-39. In the practice of the invention adsorbents other than Protein A, or in some situations, in addition to Protein A, can be used, that can adsorb CA, CA-NAB, NAB or ATAA. These adsorbents include for example, TAB (that will bind CA, CA2NAB and ATAA.) Protein G, C1q bound to anti-C1q antibody, covalently bound to matrix, C1g covalently bound to matrix (for example, Miro® sold by Fresenius HemoCare) Dextran Sulfate (for example, Selsorb® sold by Kaneka Corp.) and the adsorbent disclosed in Kuroda et al. US patent 4,627,915. Optionally, the Protein A Extracorporeal column 11 line 7. In the application of this method in accordance with the current invention an intact antibody, or an antibody fragment containing Fc fragment, and specific to a molecular species, desired to be removed, in accordance with the current invention, is bound to the protein A in the column by none covalent binding, following procedures known to those skilled in the art (see for example, E. Harlow and D Lane; Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Pub. Pp. 411-522, 1988. and Affinity Chromatography, Principles and Methods, Pharmacia LKB Biotechnology Pub. # 18-1022-29,, pp. 47-52). In accordance with the present invention the antibodies bound to the protein A in the ECA column are one or more of the following specific antibodies (SA): Antibody to TAB, antibody to CA (for example TAB) antibody to NAB, antibody to ATAA (e. g. anti idiotypic antibody), CA-NAB, NAB (anti idiotypic antibody) antibody to a tumor blocking factors and suppressor cells such as: TGF  $\beta$ , p15E, TH2 T cell epitope. In the EXAMPLE, the organism being treated is a human, the adsorption system used is the Fresenius system containing two Immunosorba® columns and a Citem 10® regeneration unit. Each

column contains 62.5 ml of Protein A , which is covalently bound to a cross linked beaded Sepharose ® matrix. Plasma is first separated from blood cellular elements, using Fresenius AS 104 Cell Separator . The plasma flow rate in the Immunosorba ® column is approximately 20-35 ml/min (flow rates can vary , depending on the individual case and can be in a range from 5 to 50 ml/min, when Immunosorba ® column is used.

Depending on the individual case and the particular column used , persons with skill in the art can determine without undue experimentation the appropriate flow rate , in the individual case. Step One above can be completed preferably from 0.1 hours to 24 hours prior to the TAB-VL or TAB-TL administration. Most preferably it will be completed between 0.15 hours to 4 hours , prior to the TAB administration. The length of step one is typically between 1 hour and 17 hours and more typically between 2 hours and 4 hours, depending on the individual case including , for example , age , weight, presence of pathological fluids in body cavities , such as peritoneum, interstitial space, pericardium and pleural cavity, as examples. It should be understood that while in the EXAMPLE, plasma is being treated, other bodily fluids can be treated, such as peritoneal fluid, lymph, cerebro spinal fluid, with access to these biological fluids achieved by methods that are well known in the art. When fluid of the Blood Circulatory System is accessed and treated , the fluid may be blood rather than plasma (see for example US patent 5,753,227, including use of encapsulated adsorbent , when blood is directly treated) . Following the adsorption STEP ONE, in the EXAMPLE, in STEP 2 , the TAB is administered intravenously. The TAB in the EXAMPLE is Hybrid of the two intact monoclonal antibodies Mab CHA-255 , specific to the hapten L-SCN-C6H4-CH2-EDTA and Mab ZCE-025 specific to CEA (C. Lollo et al. , Nuclear Medicine Communications, Vol. 15, pp. 483-491, 1994) The VL is 111 In-NBE-EDTA , which is bound none covalently to antibody binding site of Mab CHA-255. The hybrid of Mab CHA-255 and Mab ZCE-025 is prepared by using the method described by Lollo et al. Supra, except that instead of the hybrid F(ab')<sub>2</sub> , a hybrid of the intact antibodies (which is the preferred species used) is the hybrid utilized. Alternatively it is possible to use hybrid of one intact Mab with the Fab fragment of the second Mab thus including in the hybrid Fc piece of at least one antibody molecules , to enable binding to Protein A and Protein G , used as the adsorbent in the extracorporeal column . US patent 5,753,227, that is incorporated in this application by reference reads in column 10, line 51 to column 11 , line 8: "When affinity adsorption is used in accordance with the present invention for the adsorption of antigens or haptens. such as adsorption of LDL or oxidized-LDL in the treatment of atherosclerosis or adsorption of rheumatoid factor (autoantibodies to Human IgG) in the treatment of rheumatoid arthritis. for example, the application of the analytical method of J. Goding et al.. J. Immunological Methods. vol. 20, 1978. pp. 241-53, to extracorporeal affinity adsorption in accordance with the present invention. is a general method for the removal of antigens or haptens from the body. It should be clearly realized that any antigen or hapten can be removed from the body in accordance with this invention. In accordance with this method first Protein A or genetically engineered Protein A peptide (R. Lindmark et al.. supra) is covalently bound to any of the matrixes described in the current invention. for example Sepharose 4B CL The antibody specific to the antigen. for example monoclonal anti-body specific to LDL is added to the Sepharose-bound Protein A. It binds to Protein A through its Fc part. and its Fab antigen binding part is available to bind the antigen (LDL). The matrix bound Protein A-LDL-antibody is incorporated in the ECA column as described in the foregoing examples for the treatment of atherosclerosis, Clearly it is possible to use Protein G instead of Protein A in this system."

The adsorbent used in the CEA column may also be, Mab CHA-255 bound to Protein A or Protein G, through Fc of the Mab, EDTA that is covalently bound to the matrix, preferably through a spacer arm having a length of between 5-20 carbon atoms. Alternatively the EDTA can be conjugated to IgG that will bind to the Protein A through The IgG Fc. The EDTA will adsorb Free 111-In, released in the blood circulatory system.

A modification of the method is to use liposome incorporation of VL in accordance with WO 96/37516. The incorporated ligand may be TAB-VL (For example, Mab ZCE-025-Mab CHA-255-NBE-EDTA-111 In) or it may be any other 111 In containing species, such as EDTA-111 In and target the liposome to the tumor, by binding to the wall of the liposome, covalently, or by ligand (non-covalently) Mab ZCE-025, or its fragment that will target the liposome to the tumor.

#### EXAMPLE 2

Is identical to Example ONE above, except that the STEP 3 of ECA of TAB-bound 111 In NBE-EDTA-111 In, or free 111 In is omitted.

#### EXAMPLE 3

Is identical to EXAMPLE 1, except that prior to STEP 1 of ECA, in order to increase the affinity of the adsorbent Protein A, used in the EXAMPLE, monoclonal antibodies specific to the ATAA, (anti idiotypic antibodies to the FV binding site of ATAA), are administered to the treated subject. The production of monoclonal Anti Idiotypic antibodies to ATAA, are well known to those skilled in the art, as ATAA is a complete antigen. While use of monoclonal antibodies, preferably, chimeric or humanized antibodies are preferred, polyclonal antibodies can be used instead. Instead of administering anti ATAA antibodies, Cold TAB, e.g. TAB that is not bound to a VL or TL, can be administered, instead. In either case the production of ATAA-Anti-ATAA or ATAA-TAB complexes will increase their adsorption by Protein A, hence their effective removal by ECA. It is realized that should TAB be administered it will also inherently have affinity to and bind CA as well as CA2NAB.

#### EXAMPLE 4

Is identical to EXAMPLE 1 except that in order to enable the extracorporeal adsorption in STEP 1 of free circulating tumor antigen (CA) that are not bound to NAB and therefore can not be adsorbed by the Protein A used as adsorbent (as well as Protein G or C1Q, when these are used as adsorbents) as well as enable the more effective adsorption of ATAA, prior to step 1 of ECA, TAB (e.g. Antibodies specific to CA) is administered (intravenously, intraperitoneally or by other route, depending on the individual case). TAB will also bind to ATAA (as the Antigen of ATAA) producing ATAA-TAB complex, thus enhancing the adsorption of the ATAA by the Protein A Protein G or C1q adsorbent in the ECA column. These TAB antibodies are preferably monoclonal antibodies, preferably chimeric or humanized antibodies, but can be polyclonal antibodies. The amount of antibody administered can be determined by those skilled in the art, depending on the individual case and will generally be between 0.1 Mg/Kg to 2 mg/Kg, Preferably 1 mg/Kg. The time interval between completion of this antibody administration and STEP 1, is relatively short, in order to reduce to minimum the access of unlabeled TAB to the tumor, thus reducing the amount of unlabelled TAB bound to TA, and reducing the competition of unlabelled TAB, with the Labeled TAB. In view of

the fact that when unlabeled TAB is administered , such as by intravenous injection , it will have immediate access to CA in the BC , but a relatively delayed access to the tumor site , the time delay between completion of administration of unlabeled TAB and the initiation of STEP 1 ECA , will generally be between 5 minutes and 6 hours, preferably between 10 minutes and 2 hours. The time will depend on the individual case and can be determined by those skilled in the art without undue experimentation.

#### EXAMPLE 5

Is similar to EXAMPLES 1-4 , except that in order to enable adsorption of free NAB , by Protein A, Anti Idiotypic antibodies specific to NAB are administered prior to STEP 1 of ECA.

#### EXAMPLE 6

Is similar to EXAMPLES 1-5, except that in order to enable adsorption of tumor immunity molecular suppressors , (Such as TGF $\beta$  and p15E) or tumor immunity cellular suppressors ( such as TH2 suppressor cells), antibodies to the suppressor molecules and /or suppressor cells (e. g. In the example , antibodies to Th2 epitopes ) is administered , preferably, prior to STEP 1 ECA.

#### EXAMPLES 7-12

Are similar to examples 1-6 except that the targeted ligand is a the TL Adriamycin , which is administered in accordance to EXAMPLE 2 of WO 96/37516, incorporated herein by reference.

#### EXAMPLES 13-19

Are similar to Examples 7-12 except that the Adriamycin is covalently bound to an intact Mab specific to the Tumor Antigen (TA) alpha-fetoprotein (AFP) and the method is in accordance with EXAMPLE 3 of WO 96/37516, incorporated herein by reference.

#### EXAMPLE 20 TO 26

The TAB is intact antibody ZCE-025 (see example 1) specific to CEA . It is directly Iodinated with <sup>131</sup>I , using the Chloramine-T (CT) method following the procedure of J. A. Carrasquillo et. al. Cancer Treatment Reports, Vol. 68, No 1, pp. 317-328, January 1984.

The TAB-<sup>131</sup>I is administered in a dose containing 5 to 400 mCi radioactivity. The corresponding amount of Iodinated Mab is 0.65-52 mg. The other parameters of Tab administration are identical to those in EXAMPLES 1-6 In the above examples the <sup>131</sup>I TAB is used for TREATMENT.

The dose used for diagnostics is 5 to 15 mCi . The procedures for adsorption of CA, and/or CA-NAB and/or NAB and/or TAAA and/or TGF $\beta$  and/or p 15E and/or other tumor suppression factors are identical to those described in EXAMPLES 1-6.

In all the EXAMPLES 1-26, wherein prior to STEP 1 ECA, the treated organism is administered a species (TAB, Antibody to TAA, Antibody to NAB Antibody to a Tumor Suppression Factor, such as TGF $\beta$  and p 15 E, for example) aimed at production of a complex between the administered species and a Targeting-Inhibitor Species or a Tumor Immunosuppressor species. Rather than administering the species to the treated subject, the species (such as the TAB, antibody to TAA etc. Supra), which antibodies comprise an Fc piece (preferably the antibodies are intact monoclonal antibodies) The species is bound to the Protein A adsorbent in the ECA column, rather than being administered to the organism, following the general method of using ECA with Protein A or Protein G bound to a specific antibody, in accordance with US patent 5,753,227 incorporated by reference

#### EXAMPLES 27 to 52

The procedure for the adsorption of CA and/or CA-NAB and/or NAB and/or TAAA is identical to examples 1-26. The TAB is identical to the TAB of EXAMPLES 20-26, except that the TAB is treated in accordance to the procedures disclosed in US patent 6,251,394, for the labeling of the TAB for post TAB administration adsorption., This patent is incorporated herein in its entirety, by reference. The labeling of TAB is preferably with Biotin.

The TAB may contain any of the Therapeutic or Diagnostic ligands described in the above patent (as well as those described in the current patent application in its entirety, including, but not limited to EXAMPLES 1-26 above and those disclosed in PCT WO 96/37516 . ).

The subject being treated for therapeutic or diagnostic purposes is a human subject with a malignant melanoma tumor which is positive for the p97 surface glycoprotein antigen. The TAB is the monoclonal antibody 96.5 specific to p97. The TAB is labeled with <sup>131</sup>I and conjugated to Biotin in accordance with US patent 6,251,394. See column 8, line 66 to column 9, line 19:

"The monoclonal antibody was the 96.5 (mouse IgG2a) specific for p97, a cell surface glycoprotein with the molecular weight of 97,000 present on 60-80% of human melanoma. The tumor model has been described in detail (Ingvar. C. et al. Nucl. Med 30. 1989, 1224). 2. Conjugation and Labeling of Monoclonal Antibodies. The monoclonal antibody 96.5 (330 ug) was labeled with s 37 MBq iodine-125 (<sup>125</sup>I), using the Chloramine-T method. By elution on a Sephadex G25 column (Pharmacia PD10) the fraction containing the labeled protein was collected and used for the conjugation. The labeling efficiency of the <sup>125</sup>I 96.5 was around 70%. The radiolabelled monoclonal antibody was conjugated with biotin by mixing 500 ug of antibody with 41 ug of N-Hydroxysuccinimido-biotin (NHS-biotin) in 0.1M NaHCO<sub>3</sub>, 0.15M NaCl with 10% DMSO. The mixture was incubated for 1 h at room temperature, followed by overnight incubation at 4° C. The <sup>125</sup>I McAb-biotin conjugate was separated from free biotin-reagent by gelfiltration on a Sephadex G25 column, equilibrated with PBS (10 mM Sodium phosphate, 0.15 M NaCl, pH 7.3). The conjugate was stored at 4° C until used.

The dose administered to the subject is the same as in examples 20-26 (5-400 mCi, 0.65-52 mg TAB). 4 to 48 hours, preferably 12 to 24 hours after injection of the TAB, the subject is treated by passing his blood through an Avidin adsorption column, preferably Mitradep® column produced by Mitra Products, Inc.

The length of adsorption is in most cases between 1 hour to and 10 hours, most preferably, between 2 hours and 4 hours and depends on the individual case, including body weight of the subject and dose of TAB, and can be determined by a person skilled in the art with out undue experimentation. The volume of plasma treated is between 1 and 6 plasma volumes, most preferably between 2 and 4 plasma volumes. The flow rate is between 10-50 ml/min. A scintillation camera is used for imaging, when imaging is desired."

In the step of removal of CA, CA-NAB, ATAA, NAB, TGFβ, P15E and other molecular and cellular Tumor Suppression Factors (TSF), rather than using Protein A as adsorbent, Protein A bound to a specific antibody to the TSF (TSF molecules and/or cells TH2 suppressor cells epitopes, for example), in accordance with the general ECA method, wherein the adsorbent is Protein A bound to a specific antibody, as disclosed in US patent 5,753,227, incorporated by reference. Protein A bound to specific antibody, can be used instead of, or in addition to free Protein A adsorbent (e. g. Protein A unbound to specific antibodies.) Alternatively to the above option, in the step of removal of CA, CA-NAB, ATAA, NAB and other molecular and cellular TSF, a FIRST affinity adsorbent, other than Protein A or Protein G, can be used for binding to the FIRST affinity adsorbent a SECOND affinity adsorbent, specific to the TSF, in accordance with the general principle of US patent 5,753,227, in particular column 10, line 51 to column 11, line 80:

"When affinity adsorption is used in accordance with the present invention for the adsorption of antigens or haptens. such as adsorption of LDL or oxidized-LDL in the treatment of atherosclerosis or adsorption of rheumatoid factor (autoantibodies to Human IgG) in the treatment of Rheumatoid arthritis. for example. the application of the analytical method of J. Goding et al.. J. Immunological Methods. Vol. 20, 1978. pp. 241-53. to extracorporeal affinity adsorption in accordance with the present invention. is a general method for the removal of antigens or haptens from the body. It should be clearly realized that any antigen or hapten can be removed from the body in accordance with this invention. In accordance with this method first Protein A or genetically engineered Protein A peptide (R. Lindmark et al.. supra) is covalently bound to any of the matrixes described in the current invention. for example Sepharose 4BCL. The antibody specific to the antigen for example monoclonal anti-body specific to LDL is added to the Sepharose-bound Protein A. It binds to Protein A through its Fc part, and its Fab antigen binding part is available to bind the antigen (LDL). The matrix bound Protein A-LDL-antibody is incorporated in the extracorporeal immunoabsorption (affinity adsorption) treatment column as described in the foregoing examples, for the treatment of Atherosclerosis. Clearly it is possible to use Protein G Instead of Protein A in this system."

For example the FIRST adsorbent in the ECA column is Avidin or Streptavidin to which is bound a SECOND specific adsorbent, comprising Biotinylated antibody to TSF. The use of Avidin-Biotin

combination, wherein the FIRST adsorbent is Avidin, was proposed by J Tennval et al. Cancer Suppl. Vol. 80, number 12, pp.2411-2418, December 15, 1997, for the removal of ATAA, by adsorbing to the Avidin in the ECA column Biotinylated TAB. In accordance with the current invention, this approach can be utilized to remove any molecular or cellular TSF, by binding to the Avidin in the ECA column one or more Biotinylated antibodies specific to one or more molecular or cellular TSF. It should be realized that the general method of US patent 5,753,227, utilizing a SECOND specific adsorbent, bound none covalently (e.g. bound by ligand) to a FIRST adsorbent that is covalently bound to a matrix, is not limited to Protein A-specific antibody or to Avidin-Biotinylated specific antibody pairs. Various pairs of affinity ligands can be used as the FIRST adsorbent and the SECOND adsorbent. The First adsorbent, for example, is an antigen or an hapten covalently bound to a matrix. For example, Dinitrophenyl (DNP) bound to a matrix, preferably through a spacer, or a carrier molecule, such as albumin (e.g. albumin-DNP conjugate bound to the matrix). The SECOND adsorbent can then be a hybrid antibody specific to both DNP and the TSF. The first adsorbent can be an antibody to DNP and the SECOND adsorbent an antibody to TSF covalently bound to DNP. The FIRST adsorbent can be an enzyme bound covalently to the matrix in the ECA and the SECOND specific adsorbent will then be an enzyme substrate or enzyme inhibitor conjugated to an antibody specific to TSF (or to another molecular or cellular species targeted for removal.) Similarly any of other known affinity pairs such as, for example, those listed in US patent 6,251,394, column 7, lines 54 to 67, can be used. Such Specific Adsorption methods using a FIRST and SECOND adsorbents can be used not only in ECA treatment, but also in adsorption-based purification or diagnostic methods, to remove any molecular or cellular species from a fluid, including but not limited to biological fluids.

#### EXAMPLE 53-56

is identical to EXAMPLES 5-8 except that the TL is the anti cancer drug Calicheamicin. The subject treated is a human being having a Acute Myeloid Leukemia positive for the CD33 antigen. The TAB is Recombinant Engineered Human Anti-CD33. The TAB is conjugated to the Calicheamicin in accordance to L M. Hinman et al., Cancer Research, Vol 53, pp. 336-3342, July 15, 1993. 3336-3342, The dose of administered TAB-Calicheamicin conjugate is 6-9 mg protein/ m2 (E L Sievers et al., Blood Vol. 93 (11), June 1 1999).

#### EXAMPLES 56-60

Are identical to EXAMPLES 1-8, except that the Targeting molecule, in these examples is a none immunologic OTP, the hormone peptide Somatostatin. The treated subject is a human being having a cancer with high concentration of Somatostatin Receptors, as determined by biopsy. (C. Casini Raggi et al. Clin. Cancer Res. Vol. 8 (2), PP. 419-427, Feb 8, 2002.) Adriamycin is conjugated to the Somatostatin in accordance with A. Nagy et al. Proc. Natl. Acad. Sci USA, Vol 95, pp. 1794-1799, 1998, The dose of the conjugate is calculated to contain 30mg to 75mg Adriamycin/ m2 body surface. The post administration of Somatostatin-Adriamycin conjugate clearance from BC and HC is done with an ECA column containing antibody to Somatostatin bound to Protein A. Alternatively, the Somatostatin is conjugated also to Biotin in accordance with C M eppler et al. J. biol Chem, Vol 267 (22), pp. 15603-12, August 5, 1992 and the conjugate administered to the subject is Biotin-Somatostatin-Adriamycin and the ECA is done with an Avidin column, for



example , Mitra ® ECA column. The subject is treated after post conjugate administration so as to treat 1-5 plasma volumes , ECA is started 1-48 hours after Biotin-Somatostatin-Adriamycin is administered , preferably 2-24 hours . The length of post conjugate administration is 1-4 hours. The flow through the column is 20-50 ml/min.

#### EXAMPLES 61-64

Are identical to EXAMPLES 56-60, except that the subject being treated is administered a Yttrium-90 labeled Somatostatin analog prepared according to A Otte et al. The lancet, Vol 351 , pp. 416-417, February 7, 1998. The dose of the conjugate is 25 mCi to 200 mCi.

#### EXMPLE 65

A conjugate of Avidin with Mab ZCE-025 ,which is specific to CEA antigen is prepared according to H. P. Kalofonos et. al. : The Journal of Nuclear Medicine , Vol. 31, No 11, pp. 1791-1796, 1990. , except that the Mab ZCE-025 is substituted for Mab HMFG1. The subject being treated is having a tumor positive to the CEA antigen and has circulating CEA antigen in his blood. As determined by standard methods known in the art and available commercially . In STEP 1: The subject is administered intravenously (1-4 min) 0.1 mg protein/Kg to 2 mg protein/Kg of the Mab-Avidin conjugate. In STEP 2: The subject is treated with ECA with a Biotin Adsorbent Column (BAC) . The BAC ECA starts 0.5h to 48 hours , preferably 2-24 hours after the administration of the Mab-Avidin conjugate. 1-4 volumes of plasma are treated . The length of the ECA is 1-4 hours . The flow rate is 20 ml/min to 50 ml/min. For its use as the adsorbent in the ECA Biotin is covalently bound to a macromolecular carrier, such as albumin , using the method described in US patent 6,251,394, except that the albumin is substituted for the antibody. Albumin labeling with Biotin can also be done , following the method described in Harlow and Lane Supra, pp. 340-341, substituting the HAS for antibody. The Albumin-Biotin is covalently bound to Cyanogen Bromide Sepharose 4B beads available from Pharmacia. As an alternative to binding of Albumin-Biotin to Cyanogen Bromide activated Sepharose , The binding of the Biotnylated Albumin to Sepharose can be done by using Avidin-Biotin binding The albumin is biotinylated , so as to have sufficient Biotin moieties conjugated to the Albumin ,thus leaving sufficient number of Biotin molecules in the ECA column to be available to bind to Avidin in the Mab-Avidin conjugate. In STEP 3: The subject is given intravenously 0.5-10 mg protein of the conjugate Biotin -Human Serum Albumin(HAS)-131 I in 1-5 ml volume of 8.4% Sodium bicarbonate. Human Serum Albumin is directly Iodinated with 131 I according to E. Harlow and D. Lane : Antibodies A Laboratory Manual, Cold Spring harbor Laboratory pub. , pp.324-329, except that Human Serum Albumin is substituted for the antibody for the direct Iodination. The preferred Iodination is by the Chloramine T method (E Harlow and D Lane Supra, 328-329.)

In STEP 4: 0.5-48 hours , preferably 2 hours to 24 hours following the administration of the Biotin-HSA-131 I- conjugate , the subject is treated by ECA column ,that includes Avidin as the adsorbent , Preferably the Avidin CEA column is the Mitra ® ECA column. Length of ECA is 1-4 hours, Flow rate in the ECA is 20-50 ml/min.

The purpose of Steps 1 and 2 is to remove CA, CA2-NAB and TAA from the BC and HC and from the Interstitial Fluid of TC, by specifically labeling CA, CA2-NAB and TAA, with the TAB-Avidin conjugate and adsorbing the complexes TAB-Avidin-CA, TAB-Avidin-CA2-NAB and TAB-Avidin-TAA by adsorption to the Biotin adsorbent in the Biotin ECA column. The Mab in the TAB-Avidin conjugate can be an intact antibody, antibody fragment, including synthetic fragment, and fragment produced by genetic engineering techniques. In addition to the removal of CA, CA2NAB and TAA, removal of any other molecular and/or cellular species can be accomplished by the labeling of the species with a conjugate of Avidin that is conjugated to an antibody to the species, such suppressor species include: NAB, CA-NAB2, Transforming Growth Factor beta (TGF $\beta$ ) p15E factor, Interleukin 10 (IL-10), Prostaglandin E2 (PGE2), Mucin, Suppressive E Receptor (SER), Immunosuppressive acidic protein (IAP) and adhesion molecules. (K E Hellstrom and I Hellstrom, Encyclopedia of Immunology Supra and C Botti et al. Int. J Biol. Markers, Vol. 13 (2), pp. 51-69, 1998.).

The use of a labeled affinity targeting molecule can be utilized by administration to the subject being treated to affinity label any molecular or cellular species, in particular in the BC but also in the HC and the Interstitial fluid component of the TC, provided that the species targeted for affinity labeling is in equilibrium between the BC, HC and TC compartment (unless the removal is desired only from the BC or from the other treated biological fluid compartment, such as peritoneal fluid CSF or lymphatic fluid, when this fluid is treated in the ECA device, when equilibrium with HC and TC is not required. It should be realized that usually, these species will be in a concentration equilibrium between the various compartments). With respect to the species that it is desired to remove in the treatment (or diagnosis) of cancer, in addition to what was listed above: Cellular species include TH2 suppressor T cells that suppress the immune destruction of tumor cells. It will be realized that the disclosed "labeling-based adsorption", will have applications other than in the treatment of cancer, to remove any endogenous or exogenously administered or invading cellular or molecular species, such as auto antibodies in the treatment of autoimmune disease, sepsis associated factors, such as Tumor Necrosis Factor, Leukotrienes, Bradykinin and Interleukin 2, in the treatment of sepsis. Viruses and bacteria as well as protozoa in the treatment of infectious diseases. Toxins: e.g. Tetanus toxin, Botulinum toxin, for example. Other utilizations include: Affinity labeling for ECA of specific cellular and/or molecular species that are collected or harvested for therapeutic use, in the same subject, from which they are removed, or in another subject: For example, removal of specific T cell population, for in vitro treatment followed by readministration to the subject, in the treatment of cancer, e.g. In vitro stimulation of harvested T cells by treatment with Lymphokines, in LAK or TIL cell treatment of cancer (See for example J. J. Sussman et al., Ann Surg oncol, Vol 1 (4), pp. 296-306, 1994 and S A Rosenberg et al., Science, Vol. 233(4770), pp. 1318-21, Sept 1986.) The only requirement for such affinity adsorption harvesting is the availability or the ability to produce specific affinity labeling reagent that is specific to the target molecular or cellular species, and the ability to conjugate the targeting species to an affinity marker, such as Avidin (or Biotin), for example. (It would be obvious that the method can be modified, by for example, the use of Biotin for Affinity labeling and use of Avidin as adsorbent in the ECA, or the use of affinity pairs other than Avidin Biotin, for example: anti hapten antibody - hapten, Enzyme - substrate and the likes. One significant advantage of the proposed affinity labeling - affinity adsorption (ECA), is that it can be possible to use one single ECA device (Avidin-ECA, Biotin-ECA, for example) to remove many

different species form the subject, by using different specific affinity labels targeted to the species to be removed and adsorbing them on the single device used for the ECA step of the method. Altern The different species can be removed at different times or at the same time .

For example: If the species is TAA it can be targeted by TAB-Avidin or by Anti-idiotypic antibody to TAA and remove by a Biotin-ECA. If , the species is Oxidized LDL it can be affinity labeled by administering a conjugate of Avidin-Antibody to oxidized LDL and removed by ECA with Biotin as the adsorbent. Pairs of affinity labels and their counterparts are known in the art and are disclosed , for example, in US patent 6,251,394 , column 6 line 7 to column 8 line 53. Many specific ligands in the Avidin-specific ligand conjugate are known and many are available from commercial sources. Generally , such specific ligands are known by , or can be produced by persons with ordinary skill in the art, following established methods and without undue experimentation. For example , if the specific targeting ligand is a Mab, many of the molecular and cellular species , that are affinity targeted for removal have known Mabs that are specific to them and many of them are available commercially. For example Mabs to CEA and other tumor antigens mentioned in the current application. Alternatively , such Mabs can be produced, without undue experimentation by those skilled in the art, by use of Hybridoma Mab production techniques. As an alternative to administering a affinity labels to the subject , the TSF affinity labels can be incorporated in the ECA column , utilizing an ECA method that incorporates as adsorbents a FIRST and SECOND adsorbents. For example the FIRST adsorbent may be Avidin or Protein A covalently bound to the matrix in the ECA column and the "SECOND" adsorbent may be a plurality of biotinylated antibodies (when Avidin is the FIRST adsorbent, or untreated antibodies (when Protein A is the FIRST adsorbent) that are specific to at least two TSF species. Use of Double Stage Labeling of a tumor for Radiolabeling a cancer was reported in H. P. Kalofonos et al. , The Journal of Nuclear Medicine , Vol. 31, no 11, pp. 1791-1796, Nov. 1990. The authors labeled a lung cancer with a conjugate of a Mab specific to the cancer antigen, that was conjugated to Streptavidin and administered Biotin- 111 In to radio-label the tumor for diagnostic imaging. The authors did not disclose or hinted to the possible use of such labeling for the ECA of molecular and cellular species . Matrixes other then Sepharose can be used to produce matrix-biotin conjugate for use in ECA. For example G. Paganelli et. al. Disclose the production of biotinilated Nitrocellulose and biotinilated Polystyrene (G. Paganelli et al. Int J. Cancer Suool 2, pp. 121-125, 1988.).

Avidin ECA column can be used with whole blood rather then plasma, thus simplifying the adsorbition as disclosed by J. Tennvall et al. Cancer Vol 80, No 12 (suppl.) pp. 2411-2418 Dec 15, 1997.

Following ECA on the Biotin adsorbent Column, the subject is administered a VL or TL conjugated to Biotin (it should be realized that the use of Avidin and Biotin in the example can be reversed; with the sequence of : Step 1: Administration of Biotin-TAB, Step 2: ECA on Avidin column Step 3: Administration of TL-Avidin or VL-Avidin conjugate. Step 4 ECA on a Biotin column. It would also be realized that affinity pairs other then Avidin biotin can be used instead of Avidin- Biotin pair such as , for example: Hapten -Anti hapten antibody, Enzyme-substrate, Enzyme-Enzyme inhibitor.) The VL-Biotin conjugate can be Biotin- 111 In and is prepared and administered to the subject according to Kalofonos et al. Supra: Biotin covalently conjugated to Diethylenetriaminepentaacetic

acid (DPTA) is obtained from Sigma chemical comp. St. Louis, Mo and chelated to <sup>111</sup>In as described in Kalafonos et al. Supra.

#### Example 66

In STEP 1 , the subject is a human with CEA positive cancer , as described in Example 65 is administered a conjugate of Avidin with Mab ZCE-025 specific to CEA.

In STEP 2, The subject is treated with ECA , incorporating biotin as the adsorbent. The purpose of this step is to remove CA, C2 -NAB and TAA.

In STEP 3, An anti-cancer drug , including ,but not limited to one or more of the anticancer drugs disclosed in PCT WO 96/37516 as well as the anticancer drugs disclosed in US patent 5,527,528, incorporated herein by reference is incorporated in a liposome prepared as described in US patent 5,527,528 , in particular , example 3 , for the preparation of Avidin coated liposome omitting the last step of incubating the Avidin coated liposomes with biotinilated antibodies. Optionally the Avidin is bound to the Biotin on the liposome wall through a spacer. Suitable spacers are disclosed, for example , in K Hashimoto et al. Biochim Biophys Acta, Vol. 856 (3), pp. 556-65, April 25, 1986. The anticancer drug incorporated into the liposome in the Example is Adriamycin.

Step 4 , The subject is treated by ECA with Biotin incorporated as the adsorbent , to remove liposomes from the BC, that did not reach or attached to the cancer.

Optionally Step 2 , and/or step 3 can be omitted .

Alternatively the liposomes are Biotinilated liposomes of US patent 5,527,528, optionally with the Biotin connected to the liposome wall with a spacer arm, the Adsorbent in the ECA of Step 2 is Aivdin and the adsorbent in the ECA in step 4 is Biotin.

#### Example 67

The subject is the same as in example 66. STEP 1 he is administered Mab ZCE-025, specific to CEA conjugated to Biotin following the method of US patent 5,527,528. In STEP 2 , The subject is treated with ECA incorporating Avidin as the adsorbent. In Step 3 , the subject is administered Ricin A conjugated to Avidin (L. K. Mahal et al. Science , Vol 276, pp. 1125-1128, 16 May, 1997.)

In STEP 4. The subject is treated with ECA , incorporating Biotin as the adsorbent.

In all of the Examples wherein the administered species is Avidin conjugate of TAB, or the administered species is Avidin conjugate of a none immunologic targeting molecule, in order to remove from the treated subject , when desired any of the species that would inhibit targeting and/or species that would in general suppress the immune destruction , or none immune mechanism destruction of the tumor , such targeting inhibitors and/or tumor destruction inhibitors can be removed from the BC , HC and TC by incorporating in the ECA adsorption column one or more adsorbents that have specific affinity to the targeting inhibitor or tumor destruction suppressive

molecular and cellular species. This can be accomplished by incorporating such specific adsorbents in a single column, or in different columns, connected in parallel or in series, as disclosed in US patent 5,753,227. When the ECA incorporates Avidin as the adsorbent, The specific adsorbent added to the ECA Avidin column is a Biotin conjugate of a specific affinity ligand (such as Biotin-Mab specific to CA, and/or ATT and/or any of the other suppressors as disclosed in Hellsrom and Hellstrom, And in botti et al. Supra.) When the adsorbent in the ECA column is Biotin, the Specific adsorbent added is Avidin-Mab specific to the CA and/or ATT, or other targeted inhibitors or tumor destruction cellular or molecular species as above.

#### EXAMPLE 68

In any of the Examples 1-67, in the step of removal of specific species from the blood circulatory system or from other biological fluid, such as, peritoneal and CSF, the specific Protein A -Specific Intact antibody adsorbent ligand, can be used for the removal of any substance from a biological fluid source, and for the application of the current invention, can be used for the adsorption- removal of any chemical species that may interfere with targeting, such as CA, CA-NAB, NAB, ATAA, or any molecular or cellular species that suppresses the Immune Destruction, or None Immune destruction of the tumor. The specific adsorbent is based on the method disclosed in US patent 5,753,227. Said patent is incorporated in the current application in its entirety. In accordance with the current example, intact antibodies, or antibody fragments, containing Fc., wherein said antibodies or Fc containing fragments are specific to an epitope on the molecular or cellular species that is to be removed from the biological fluid and BC, HC and TC are added to a Protein A extracorporeal column, such as Immunosorba ® or Prosorba ®, or any other Extracorporeal column that contains Protein A, bound to matrix or encapsulated, as disclosed for example in US patent 5,753,227, supra. Such antibodies or Fc containing fragments, in the current example are selected from antibodies, or fragments specific to the molecular and cellular species, that inhibit targeting or suppress tumor destruction as disclosed in the current application and in Hellstrom and hellstrom and Botti et al., supra.

As disclosed in US patent 5,753,227 and also detailed in Harlow and Lane Supra ,pp. 519-523, the antibody or fragment will bind to the protein A in the ECA through the Fc of the antibody or fragment, thus producing a specific adsorbent ligand to specifically adsorb one or more of the Targeting-inhibiting molecular species, or molecular or cellular species that inhibit or suppress tumor destruction. This can be accomplished by incorporating such specific adsorbents in a single ECA column, or in different columns, connected in parallel, or in series, as disclosed in US patent 5,753,227.